# Artificial Life and Nanobiotechnology; Foundations for Autonomous Robotic Systems

**Carlo D. Montemagno, PhD**

*Roy and Carol Doumani Professor of Biomedical Engineering*
*Professor Mechanical and Aerospace Engineering*
*Chair of Academic Programs, Biomedical Engineering, IDP*
*University of California, Los Angeles*

**Workshop on Revolutionary Aerospace Systems Concepts for Human/Robotic Exploration of the Solar System**
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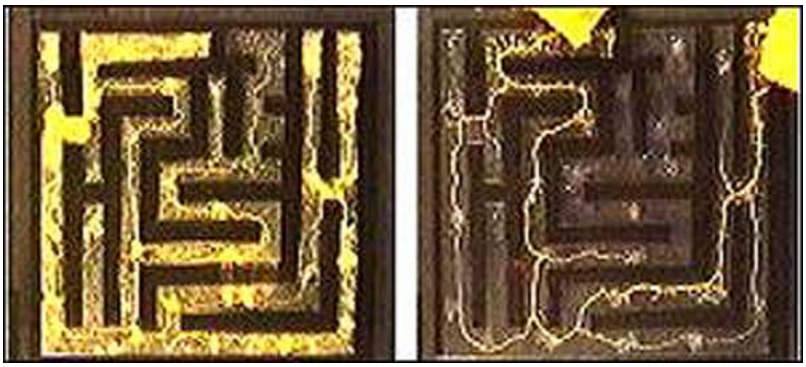
## Intelligence out of Complexity?



Slime Mold (Physarum polycephalum) before (left) and after (right) negotiating the maze (Nakagaki, Nature, 2000)

## Emergent Intelligence?

- Fully distributed Intelligence
- Stupid Agents
- Local Communication
- No “Pacemakers”
- Adaptive
  - Ants
  - Humans

## Emergent Intelligence and Nanotechnolgy

- Bottom up Strategy
- Integration at the most basic level of information and energy transduction

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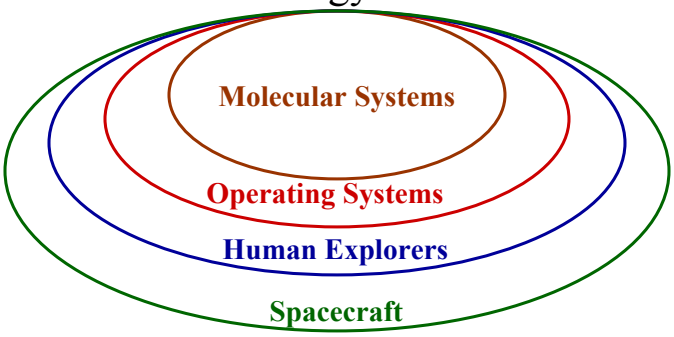
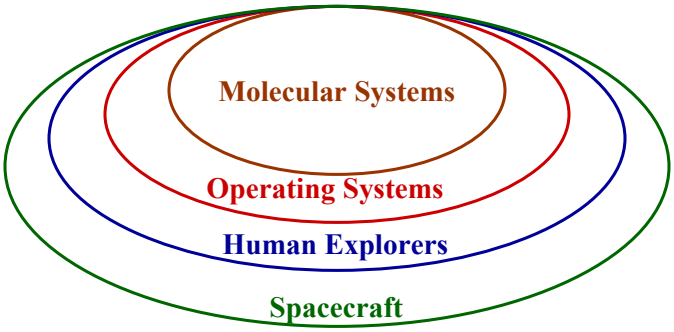
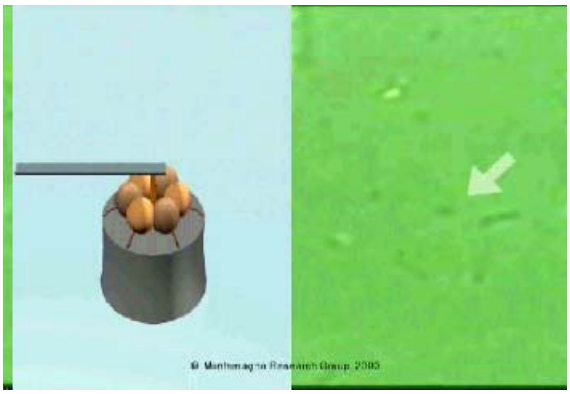


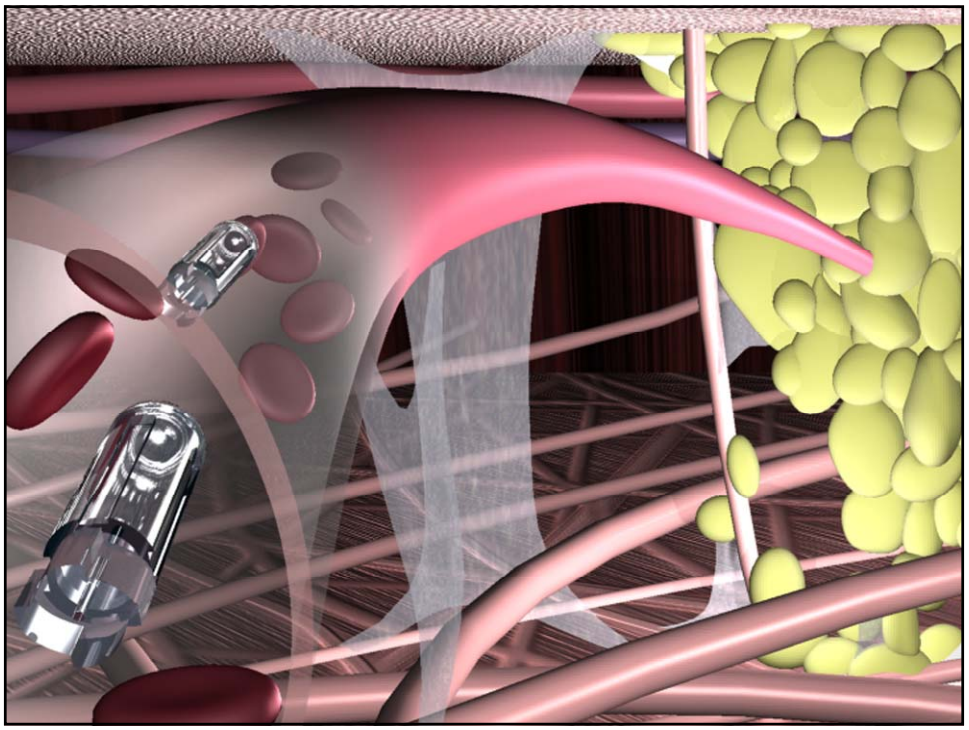

## Emergent Intelligence and Nanotechnolgy




**Mechanism for Emergent Intelligence is Nanobiologic!**

## Molecular Motor Powered Nano-propeller System

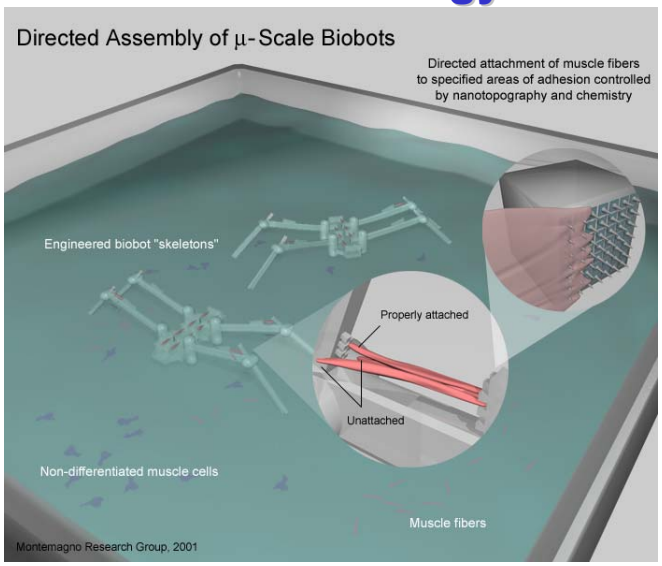




## Nano-Possibilities

## Initial Implementation: Self-Assembled Biobots

# Self-Assembly Through Nanotechnology




# Near-Term Goals

- To demonstrate the integration of *in-vitro* grown muscle tissue and fabricated structures
- To develop methods that will allow the self-assembly of muscle tissue onto fabricated devices
- To build complex mechanical systems solely powered by cultured muscles

## Biologic Tissue Actuator Development Challenges

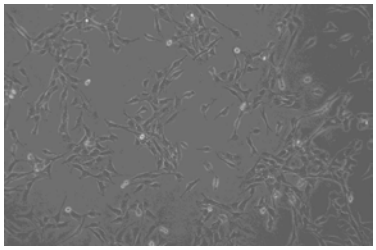
- Cell fusion into myotubes has to be controlled to attain correct size and placement for the self-assembling process
- Selective cell growth is imperative to prevent muscle tissue from growing randomly at undesired sites
- Further differentiation into tendon-like tissue is needed for anchoring the structures onto microfabricated structures

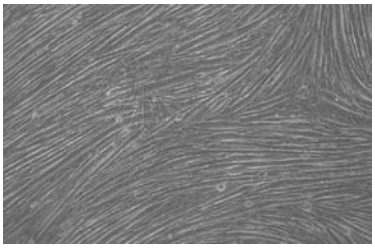
## Initial Cell Culture Tests

- Selective growth tested using a number of polymers, proteins, and inorganic substrates

| Good attachment | Good rejection |
|---|---|
| Polycaprolactone | **Polydimethylsiloxane** |
| **Polysulfone** | Polylysine |
| Polyhydroxybutirate | |
| Laminin | |
| Gelatin | |
| Au, $SiO_2$ | |

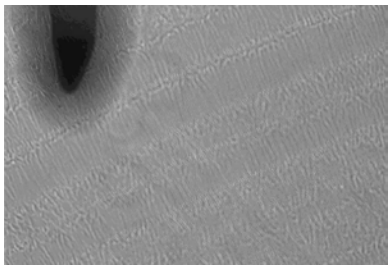
## Initial Cell Culture Results

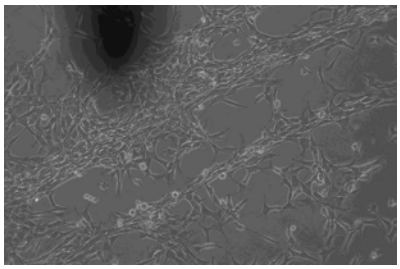




Optical micrographs of a free-growing cell culture. The picture on the left shows myoblasts just a day after the culture started. On the right we see the same culture a week later; the myotubes are already bundling together.

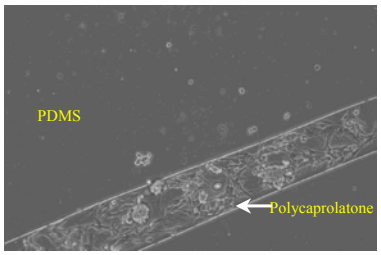
## Initial Cell Culture Results

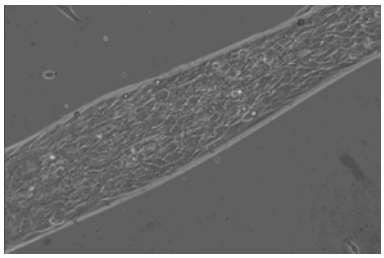




We have also explored the use of crystallization patterns that may favor cell growth. The micrograph on the left shows a polyethylene glycol pattern; on the right we see the onset of a myoblast culture that clearly follows the PEG pattern.

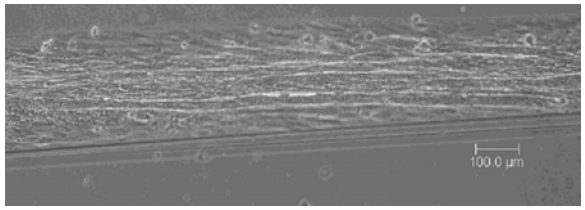
## Initial Cell Culture Results



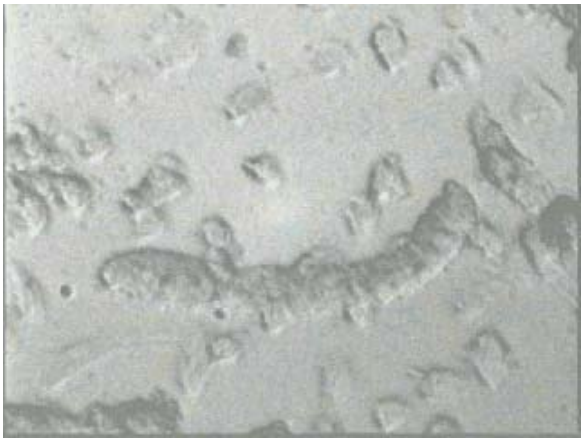
Optical micrographs show one-day and two-day confined cell cultures. The onset of line-up/fusion is evident in the latter. Also note the absence of myoblasts outside the growth area, thus evidencing good selective growth.

## Initial Cell Culture Results




After eight days the same culture shows much longer myotubes, in excess of 1 mm. No appreciable cell growth is observed outside the confinement area.

## Stimulate Muscle Myoblast



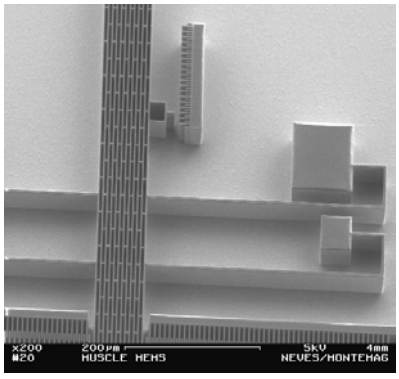
## Muscle-MEMS™ Device Challenges

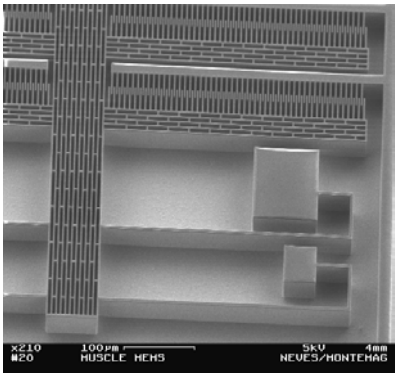
- A true three-dimensional tissue can be obtained (Dennis, 1997) by co-culturing myoblasts and fibroblasts or in microgravity environments (Pellis, 2001)
- Microfabricated structures need to be modified to direct selective tissue anchoring and optimally utilize myofibril force production.
  - nanoscale manipulation of surface topography and chemistry
  - mechanical force and spring constants need to be consistent with the performance of the muscle actuator

# Hybrid Device Integration Workplan

1. **Demonstrate integration using dissected muscle tissue**
   - Physical characterization of dissected tissues will help us design more suitable microsystems
2. **Develop selective tissue culture techniques while ensuring preserved muscle excitability**
3. **Design and fabricate microstructures that are consistent with the generated forces for**
   - **Proof of concept**
   - **Physical characterization**
4. **Build microstructures with embedded electrical stimulation**

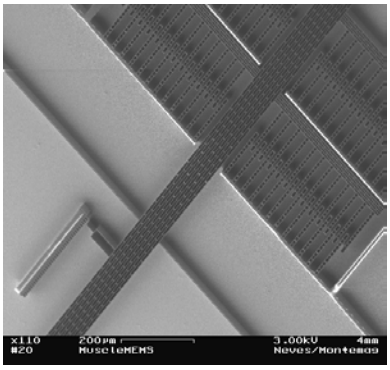
# Microsystem Design



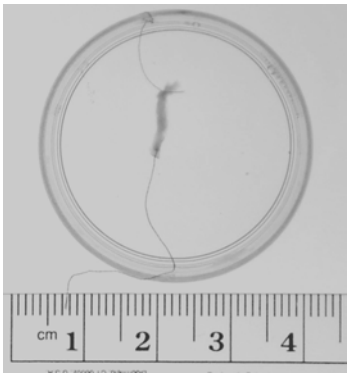

A sturdy structural design was needed to resist microsurgery procedures. The device is able to measure displacements visually (using a vernier) and electrically (by measuring capacitance changes in the comb structure)

## Microsystem Design




Subsequent changes in the choice of dissected fiber length called for a longer displacement device. A new microsystem version was then designed and fabricated. The SEM micrograph shows the trussed comb fingers employed in this design.

## Tissue Dissection

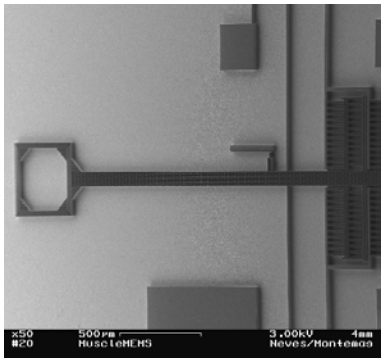


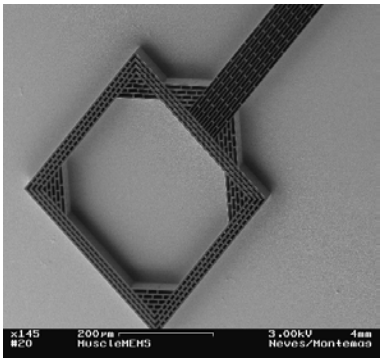

We have recently been able to isolate fine myotube bundles from dissected frogs while maintaining excitability for days. The picture shows a fiber prepared with sutures for microsystem assembling.

## Tissue Dissection

The picture shows a frog muscle fiber prepared with sutures for microsystem assembling. This bundle is approximately 1.5mm in diameter.

## Microsystem Design




The microsurgery techniques used in the assembling of the fibers onto the microsystems required large device tilting as well as more suitable anchoring structures. The SEM micrographs show the extra-long arm and the anchoring frame for suture loop-around.

## Conclusions

- We have successfully demonstrated the selective growth and differentiation of *in-vitro* muscle cell lines
- Demonstration of microsystems integration is under way using dissected muscle tissue
  - On-chip electrical stimulation will soon be incorporated in device design
- We are starting the investigation of self-assembling integration using collagen-like (P-15) impregnated microfabricated structures
- **Initiated Emergent Intelligent studies using Slime Mold as both a computational and experimental model**